ADP-Glo™ Kinase Assay Application Note

Ser/Thr Kinase Series

SIK3 Kinase Assay

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Scientific Background:

SIK3 (QSK) is a serine/threonine-protein kinase, belongs to QIK subfamily. The phosphorylation of SIK3 by LKB1 through the 14-3-3 binding enhances its catalytic activity and leads its localization to punctate structures within the cytoplasm (1). Overexpression of SIK3 promotes G1/S cell cycle progression with ovarian cancer (2). There are two sites (H331L and A1103V) were mutated at significant frequency in breast cancer. SIK3 is a novel tumor-associated antigen (TAA).

1. Al-Hakim A.K., et.al: 14-3-3 cooperates with LKB1 to regulate the activity and localization of QSK and SIK. J. Cell Sci. 118:5661-5673(2005).

2. Charoenfuprasert S, et.al: Identification of salt-inducible kinase 3 as a novel tumor antigen associated with tumorigenesis of ovarian cancer. Oncogene. 2011 Aug 18;30(33):3570-84.

ADP-Glo™ Kinase Assay

Description

ADP-Glo™ Kinase Assay is a luminescent kinase assay that measures ADP formed from a kinase reaction; ADP is converted into ATP, which is converted into light by Ultra-Glo™ Luciferase (Fig. 1). The luminescent signal positively correlates with ADP amount (Fig. 2) and kinase activity (Fig. 3A). The assay is well suited for measuring the effects chemical compounds have on the activity of a broad range of purified kinases—making it ideal for both primary screening as well as kinase selectivity profiling (Fig. 3B). The ADP-Glo™ Kinase Assay can be used to monitor the activity of virtually any ADP-generating enzyme (e.g., kinase or ATPase) using up to 1mM ATP.

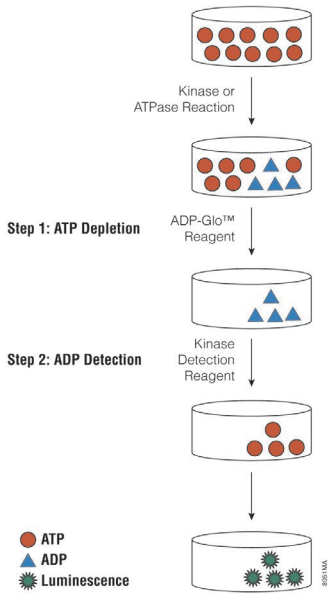

Figure 1. Principle of the ADP-Glo™ Kinase Assay. The ATP remaining after completion of the kinase reaction is depleted prior to an ADP to ATP conversion step and quantitation of the newly synthesized ATP using luciferase/luciferin reaction.

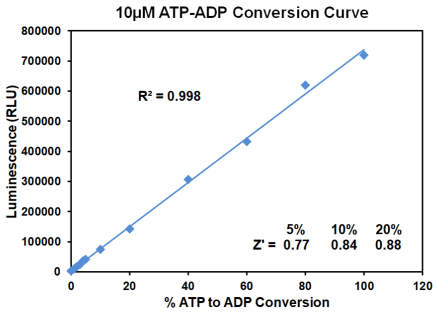

Figure 2. Linearity of the ADP-Glo Kinase Assay. ATP-to-ADP conversion curve was prepared at 10µM ATP+ADP concentration range. This standard curve is used to calculate the amount of ADP formed in the kinase reaction. Z' factors were determined using 200 replicates of each of the % conversions shown.

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The following is only a short protocol. For detailed protocols on conversion curves, kinase assays and inhibitor screening, see Kinase Enzyme Systems Protocol at: http://www.promega.com/KESProtocol

Short Protocol

- Dilute enzyme, substrate, ATP and inhibitors in 1x kinase reaction buffer.
- Add to the wells of 384 low volume plate:
  - ✓ 1 µl of inhibitor or (5% DMSO)
  - ✓ 2 µl of enzyme (defined from table 1)
  - ✓ 2 µl of substrate/ATP mix
- Incubate at room temperature for indicated time (See Figure 3).
- Add 5 µl of ADP-Glo™ Reagent.
- Incubate at room temperature for 40 minutes.
- Add 10 µl of Kinase Detection Reagent.
- Incubate at room temperature for 30 minutes.
- Record luminescence (Integration time 0.5-1 second).

Table 1. Enzyme Titration. Data are shown as relative light units (RLU) that directly correlate to the amount of ADP produced. The correlation between the % of ATP converted to ADP and corresponding signal to background ratio is indicated for each kinase amount.

Enzyme, ng	200	100	50	25	12.50	6.25	3.13	1.56	0.78	0
Luminescence	271,299	134,611	93,991	60,546	29,984	13,959	7,244	4,578	3,000	1,652
S/B	164	81	57	37	18	8	4	3	2	1
% Conversion	64	32	22	14	7	3	2	1	1	0

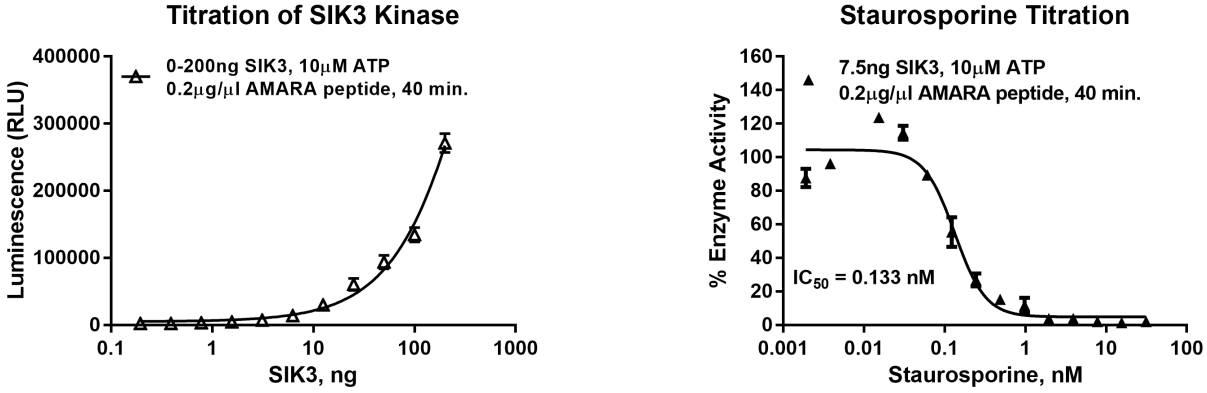

Figure 3. SIK3 Kinase Assay Development. (A) SIK3 enzyme was titrated using 10µM ATP and the luminescence signal generated from each of the amounts of the enzyme is shown. (B) Inhibitor dose response was created using 7.5ng of SIK3 to determine the potency of the inhibitor (IC_{50}).

Ordering Information:

Products	Size	Cat. #
SIK3 Kinase Enzyme System	10µg	VA7300
	1mg	VA7301
ADP-Glo™ + SIK3 Kinase Enzyme System	1 Each	VA7302